

"Nanofluidic Pre-Concentration Devices for Enhancing the Detection Sensitivity and Selectivity of Biomarkers for Human Performance Monitoring"

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Abstract: Towards enhancing the detection sensitivity of biomarkers for real-time human performance monitoring in bio-fluid media we have developed nano-device platforms for pre-concentration, separation and enhancement of binding kinetics of target species to sensing elements. We will apply these methods to key biomarkers in year 2.

Introduction: In an era of limited resources, it is essential to utilize the largest asset of the USAF, which is the air force personnel, to the full capacity through a vision based on the "augmentation of human performance". An enabling technology towards this vision is the real-time monitoring of human performance biomarkers for fatigue, vigilance, and stress that are present in biofluids such as saliva, blood, and urine. A set of such biomarkers that have been identified by the 711th Human Performance Wing at the Air Force Research Laboratory (AFRL) at Wright Patterson Air Force Base include: Orexin-A, Neuropeptide Y (NP-Y), Riboflavin, estradiol (17 β) and cytokines (IL-1 β). However, the biomarkers need to be detected at <ng/mL sensitivity levels within biofluids that contain background serum proteins. This project seeks to develop electrokinetic pre-concentration and separation strategies for enhancing detection sensitivity of biomarkers within relevant biofluid media.

Experiment: The following methodologies are being developed towards enhancing biomarker detection sensitivity within relevant biofluid media:

- (1) Dielectrophoretic separation of immobilized biomarkers on nanoparticles
- (2) Direct biomarker pre-concentration by dielectrophoresis
- (3) Direct biomarker pre-concentration by concentration polarization
- (4) Nano-slit device to enhance biomarker binding kinetics

Results and Discussion: The following summarizes our progress:

(1) Dielectrophoretic separation of immobilized biomarkers on nanoparticles: The separation of DNA aptamer capture probes bound to the biomarker of interest (NPY, for instance, in Figure 1) versus other bound or un-bound DNA aptamer capture probes (Thrombin binding aptamer, for instance) is a particularly difficult task. Since the DNA aptamer sequences are rather similar in size and biochemical properties, but differ chiefly in conformation, their separation by conventional methods is not straightforward. In this project, the differing conformation of the respective DNA aptamers immobilized on silica or gold nanoparticles causes significant differences in their net dielectric properties, which is then used to separate the nanoparticles, and hence, DNA

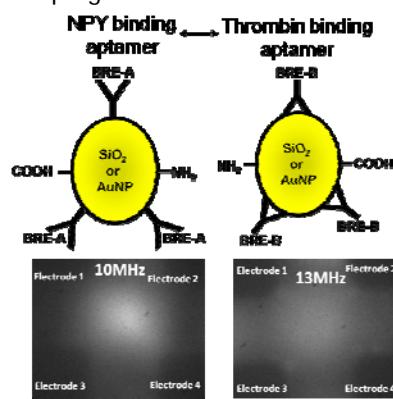


Figure 1: Dielectrophoretic separation of nanoparticle immobilized aptamers based on conformation.

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14. ABSTRACT Towards enhancing the detection sensitivity of biomarkers for real-time human performance monitoring in bio-fluid media the researchers have developed nano-device platforms for pre-concentration, separation and enhancement of binding kinetics of target species to sensing elements. They will apply these methods to key biomarkers in year 2.				
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aptamers by using dielectrophoresis methods. Dielectrophoresis (DEP) enables highly selective trapping of bio-particles based on the characteristic frequency response of the dielectric permittivity of the bio-particle versus that of the medium. **Figure 1** shows that the open charged structure of NPY binding aptamer versus closed charge structure of thrombin binding aptamer have different crossover frequency from positive to negative dielectrophoresis (10 MHz versus 13 MHz), as investigated using a quadrapole electrode geometry (lowest field at device center). While this can be applied to separate them, the separation based on differing levels of negative dielectrophoresis is not as clear as methodologies that can trap these aptamers within different sections of the device. Recently, we have developed a methodology to trap the nanoparticle immobilized with NPY binding aptamer by positive DEP, while trapping the interfering thrombin binding aptamer by negative DEP, so that they can be separated within separate sections of the device.

The enabling principle is illustrated in **Figure 2** for the modification of electric field lines by the capacitance of the capping layer around a nanoparticle. As a result of Maxwell-Wagner polarization, the nanoparticle experiences positive DEP (**Figure 2c**) at \sim MHz frequencies, causing the field lines to terminate and re-originate at the particle surface like a metallic interface, as in **Figure 2a**. For the case of the nanoparticles immobilized with the open charged structure of NPY binding aptamer, the lower capacitance of the capping layer causes no perturbation of the field lines until crossover from positive to negative DEP at \sim 10-15 MHz. On nanoparticles immobilized with the closed structure of highly-charged thrombin binding aptamer, on the other hand, the higher capacitance of the capping layer screens the electric field, as per **Figure 2b**, thereby causing the onset of negative dielectrophoresis (**Figure 2d**). This is especially apparent within media of high conductivity, where the compact double-layer causes capping layers of high capacitance. Hence, under \sim 100 kHz field conductances within media of high conductivity (> 1 mM salt), nanoparticles immobilized with the high-capacitance thrombin binding aptamer experience negative DEP (**Figure 2f**), whereas those immobilized with the low capacitance NPY binding aptamer experience positive DEP (**Figure 2e**), respectively, which can be applied to separate them. Note that the RC time constant (or frequency) for the capping layer around the nanoparticle, depends on hydrodynamic radius of the nanoparticle (a), double layer thickness (λ), and diffusion coefficient of ions (D), as given by: $\omega_{RC} = (D/a\lambda)$. Hence, nanoparticles with a highly charged and compact capping layer have a large ω_{RC} , which causes the screening of fields and negative DEP over a larger range of frequencies, extending up to 150 kHz in this case. Nanoparticles capped with a more diffuse charge structure, on the other hand, will have lower ω_{RC} , which results in a negligible frequency range for screening of fields and thereby causing positive DEP trapping only. We are currently testing this methodology for a larger range DNA aptamer immobilized nanoparticles.

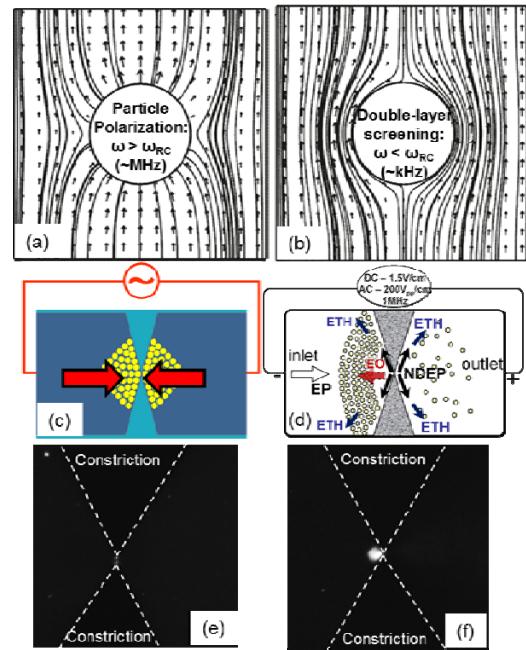


Figure 2: Electric field lines (positive to negative electrode) upon: (a) particle polarization results in positive DEP; and (b) screening by capping or double layer causes negative DEP. Device schematics for: (c) positive DEP; and (d) negative DEP (with DC bias). Experimental data for: (e) positive DEP (100 kHz – 1 MHz); and (f) negative DEP (50 – 150 kHz), due to low and high capacitance capping layers on nanoparticles, respectively.

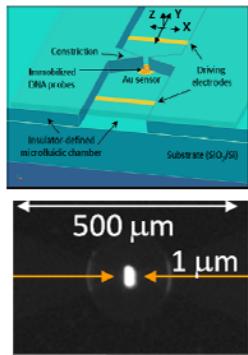


Figure 3: Constriction device for pre-concentration of DNA

1 MHz) and DC fields (1.5 V/cm) to pre-concentrate model protein target molecules, such as streptavidin, using negative dielectrophoresis in the vicinity of 1000-10000x constrictions, as per the time-lapse fluorescence images for protein trapping in **Figure 4** for devices with constrictions of ~ 50 nm (**Figure 4a-4c**) versus ~ 140 nm (**Figure 4d-4f**). At both constriction sizes, a dark circular arc corresponding

to depletion of the fluorescently labeled proteins (henceforth called the depletion zone) on the concave edge of the arc towards the constriction is apparent within a fraction of a second after the field is turned on. On the convex edge of the arc away from the constriction, we notice a strong degree of pre-concentration of the fluorescently labeled proteins (henceforth called the pre-concentration zone), especially close to the sidewall direction. It is noteworthy that at both constriction sizes, the depletion zone is established almost instantaneously and does not significantly evolve over time, while the pre-concentration zone evolves over time. The extent of the depletion zone was exponentially enhanced from $\sim 3-4$ μ m for ~ 140 nm constriction gaps to $\sim 8-10$ μ m for 100 nm constriction gaps and $\sim 20-22$ μ m for ~ 50 nm constriction gap devices. From **Figure 4g** it is apparent that upon removal of the field, the pre-concentrated protein molecules are completely dispersed across the device, demonstrating a completely reversible pre-concentration process, without any discernible aggregation. At a sub-critical DC field offset of 0.3 V/cm to the AC field under NDEP conditions (~ 200 V_{pp}/cm at 1 MHz), as shown in **Figure 4h**, the depletion zone has a very small extent (< 1 μ m) and the pre-concentration zone is wide. Furthermore, significant depletion and pre-concentration zones are not observed under AC frequency conditions that result in sub-critical NDEP, such as at 10 MHz in **Figure 4i**. In summary, the results show that an exponentially enhanced extent of protein depletion (γ) occurs upon addition of a critical DC field to the NDEP conditions, especially at smaller constriction gaps, to result in $\sim 10^4$ - 10^5 -fold protein pre-concentration. Since the exponentially enhanced depletion zone is directly correlated with the high degree of protein pre-concentration, we are currently investigating the role of enhanced ion conductance at the nano-constriction edges on the ensuing pre-concentration.

(3) Nano-slit device to reduce diffusion lengths: Following pre-concentration, we aim to enhance the kinetics of binding the biomarker to the bio-recognition element through carrying out the assay in a nano-slit device, as shown in **Figure 5**.

(2) Direct biomarker pre-concentration by dielectrophoresis: A second methodology aims to apply dielectrophoresis directly towards the pre-concentration of target molecules or DNA aptamers. In this case, sharp dielectric constrictions (500-1000-fold) are required to enhance the local field to offset the steep fall in DEP trapping forces with particle size. **Figure 3** shows that such 500-fold constrictions can be applied to pre-concentrate short fragments of single-stranded DNA, such as DNA aptamers. We are currently in the process of testing this device towards application to DNA aptamers of interest for this project, before and after binding to their target molecule.

Recently, we have applied a combination of AC fields (200 V_{pp}/cm,

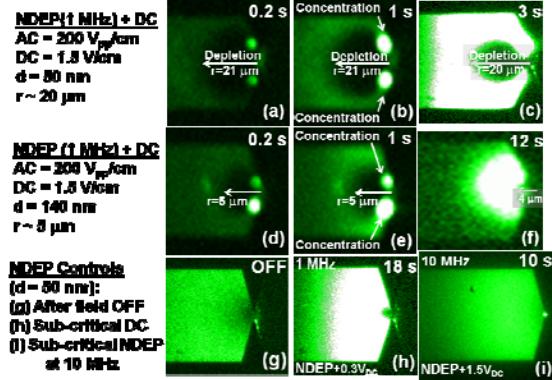


Figure 4: Time evolution of protein pre-concentration at nano-constriction devices: (a-c) 50 nm gaps; (d-f) 140 nm gaps; (g-i) varying electrical conditions.

proteins (henceforth called the depletion zone) on the concave edge of the arc towards the constriction is apparent within a fraction of a second after the field is turned on. On the convex edge of the arc away from the constriction, we notice a strong degree of pre-concentration of the fluorescently labeled proteins (henceforth called the pre-concentration zone), especially close to the sidewall direction. It is noteworthy that at both constriction sizes, the depletion zone is established almost instantaneously and does not significantly evolve over time, while the pre-concentration zone evolves over time. The extent of the depletion zone was exponentially enhanced from $\sim 3-4$ μ m for ~ 140 nm constriction gaps to $\sim 8-10$ μ m for 100 nm constriction gaps and $\sim 20-22$ μ m for ~ 50 nm constriction gap devices. From **Figure 4g** it is apparent that upon removal of the field, the pre-concentrated protein molecules are completely dispersed across the device, demonstrating a completely reversible pre-concentration process, without any discernible aggregation. At a sub-critical DC field offset of 0.3 V/cm to the AC field under NDEP conditions (~ 200 V_{pp}/cm at 1 MHz), as shown in **Figure 4h**, the depletion zone has a very small extent (< 1 μ m) and the pre-concentration zone is wide. Furthermore, significant depletion and pre-concentration zones are not observed under AC frequency conditions that result in sub-critical NDEP, such as at 10 MHz in **Figure 4i**. In summary, the results show that an exponentially enhanced extent of protein depletion (γ) occurs upon addition of a critical DC field to the NDEP conditions, especially at smaller constriction gaps, to result in $\sim 10^4$ - 10^5 -fold protein pre-concentration. Since the exponentially enhanced depletion zone is directly correlated with the high degree of protein pre-concentration, we are currently investigating the role of enhanced ion conductance at the nano-constriction edges on the ensuing pre-concentration.

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Here kinetics can be enhanced due to reduced diffusion lengths (**Figure 5a**), so that biomarker depletion is complete (**Figure 5e**) and signal saturation is reached within a few minutes for protein arrays (**Figure 5d**). Currently we use an energy free capillary pump based on 700 nm deep PDMS pillar arrays ($14 \times 14 \text{ mm}^2$) bonded to glass (**Figure 5b**), with large surface area to have enough capacity to induce capillary flow for hours to the sensor area (**Figure 5c**). The sensor arrays are constructed by contact printing for capture probe DNA or biotin patches (for streptavidin protein). In the following year, we are preparing to test this nano-slit detection platform on DNA aptamer targets of interest to the 711th Human Performance Wing.

Summary: In summary, we have thus far identified biomarkers and platforms for sensing and pre-concentration of model biomarkers to an improved sensitivity. We are in the process of applying these pre-concentration methods to biomarkers of interest to the 711th Human Performance Wing and enhancing the sensitivity of their detection systems in biofluid media.

Publications and Presentations: Following is the list of publications and presentations that resulted due to the current AOARD award:

Journal publications:

1. V. Chaurey, C.F. Polanco, C.F. Chou, N. Swami*, "Floating electrode enhanced constriction dielectrophoresis for trapping of nanoscale biomolecules in physiological media of high-conductivity", *Biomicrofluidics* (2012) 6 (1), 012806. DOI:10.1063/1.3676069. Journal Impact Factor = 3.3.

2. K.T. Liao, M. Tsegaye, V. Chaurey, C.F. Chou, N. Swami*. "Nano-constriction device for rapid protein pre-concentration in physiological media by electrokinetic force balance", *Electrophoresis* (2012), 33, 1958-1966. Journal Impact Factor = 3.3.

Conference presentations

1. Kuo-Tang Liao, Vasudha Chaurey, Mikiyas Tsegaye, Chia-Fu Chou, Nathan S. Swami; "Nanofluidics for selective protein trapping in biofluids", MicroTAS, October 2011, Seattle.
2. Nathan S. Swami, "Nano-constriction devices for electrokinetic pre-concentration of proteins", American Electrophoresis Society Meeting, Minneapolis, October 2011.

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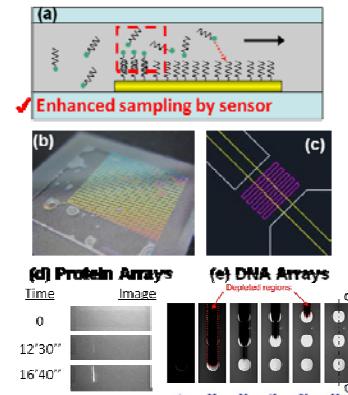


Figure 5: Nano-slit device to enhance binding kinetics.